JACC Vol. 32, No. 2
August 1998:420–6

Skeletal Muscle Response to Short Endurance Training in Heart Transplant Recipients

ELIANE LAMPERT, MD,* BERTRAND METTAUER, MD,† HANS HOPPELER, MD,‡
ANNE CHARLOUX, MD,* ARNAUD CHARPENTIER, MD,† JEAN LONSDORFER, MD*

Strasbourg, France, and Bern, Switzerland

Objectives. **We sought to examine the effects of endurance training on the ultrastructural characteristics of skeletal muscle in heart transplant recipients (HTRs) and age-matched control subjects (C).**

Background. **Deconditioning is one of the factors involved in the peripheral limitation of exercise capacity of HTRs, and training has proven to be beneficial.**

Methods. **Biopsies of the vastus lateralis muscle, analyzed by ultrastructural morphometry, and quadriceps muscle cross-sectional area, assessed by computed tomography (CT), were performed in 12 HTRs and 7 age-matched C before and 6 weeks after an endurance training program. Maximal oxygen uptake (peak $\dot{V}O_2$) was determined by an incremental exercise test. Additionally muscle biopsies were performed before and after a 6-week control period in four HTRs to check for spontaneous improvement.**

Results. **Training resulted in similar increases in peak $\dot{V}O_2$ (11% in HTRs, 8.5% in C), ventilatory threshold (23% in HTRs, 32% in C) and total endurance work (54% in HTRs, 31% in C). Volume density of total mitochondria increased significantly (26% in HTRs, 33% in C) with a predominant increase of subsarcolemmal mitochondrial volume density (74% in HTRs, 70% in C). The capillary/fiber ratio increased by 19% in C only. In the nontrained group, none of the structural markers was spontaneously modified.**

Conclusions. **Six weeks of endurance training in HTRs and C led to similar improvements of aerobic work capacity. However, the decreased muscular capillary network in HTRs remained unchanged with training. Immunosuppressive therapy might be responsible for the discrepancy between the normal mitochondrial content and the reduced capillary supply of these patients.**

(J Am Coll Cardiol 1998;32:420–6)

Exercise capacity, although improved after heart transplantation, is not totally normalized (1–3). Most heart transplant recipients (HTRs) stop exercise early because of muscular fatigue. Formerly attributed to central causes linked to heart denervation (4), and more recently, to impaired diastolic function (5), this reduced exercise tolerance seems to depend importantly on peripheral muscular factors related to physical deconditioning (6–8).

Several studies have demonstrated that exercise training for short or longer periods, from 6 weeks to 16 months, is feasible and beneficial after heart transplantation (1,2,3,9). Benefits resulting from training include increases in maximal oxygen uptake ($\dot{V}O_2$), peak exercise power output, ventilatory threshold, peak heart rate and reduced rates of perceived exertion (1,9). We have shown previously that a short endurance training program in HTRs lowers blood lactate concentrations during exercise and improves recovery by raising the efficiency with which lactate is removed (10).

In a previous study (11), we have demonstrated, by ultrastructural morphometry of skeletal muscles of nontrained HTRs 1 year after grafting, that mitochondria appear qualitatively and quantitatively normal but that the capillary network is significantly reduced. Effects of both antirejection therapy on muscle function (12,13) and deconditioning (5,7) were identified as being possibly responsible for these observations.

The purpose of this study was to assess the changes induced in skeletal muscle tissue after 6 weeks of endurance exercise: In HTRs without signs of rejection and under standard immunosuppressive treatment; in age-matched control subjects (C) subjected to a training program at the same relative intensity level and in HTRs not subjected to physical exercise, to control for spontaneous changes.

We hypothesized that exercise training leads to similar increases in mitochondria volume and capillary supply, markers of muscle tissue oxidative capacity, in HTRs and in C, but not in unexercised HTRs.

From the *Service des Explorations Fonctionnelles Respiratoires et de l'Exercice; †Service des Explorations Fonctionnelles Cardiovasculaires et Service de Chirurgie Cardiovasculaire, Hôpitaux Universitaires de Strasbourg, Strasbourg, France; and the ‡Department of Anatomy, University of Bern, Bern, Switzerland. This work has been supported in part by the Institut National de la Recherche Scientifique (INSERM) network "Activité physique, muscle et handicap," by JE 2105 "Régulations physiologiques et rythmes biologiques chez l'homme," and by Swiss National Science Foundation Grant UF-31-42449.94.

Manuscript received June 11, 1997; revised manuscript received April 6, 1998, accepted April 22, 1998.

Address for correspondence: Dr. Eliane Lampert, Service des Explorations Fonctionnelles Respiratoires et de l'Exercice, Hôpitaux Universitaires de Strasbourg, 67091 Strasbourg Cedex, France. E-mail: Eliane.Lampert@chru.strasbourg.fr.

0735-1097/98/$19.00
PII S0735-1097(98)00227-7

Abbreviations and Acronyms

C = age-matched control subjects
CT = computed tomography
HTRs = heart transplant recipients
TGF-β = transforming growth factor-beta
VEGF = vascular endothelial growth factor
$\dot{V}O_2$ = oxygen uptake

Methods

Patient population and normal subjects. Sixteen patients (15 men, 1 woman) with clinically stable orthotopic cardiac transplantation were examined at a mean time of 10 ± 3 months after being grafted. Before surgery, patients were classified according to the New York Heart Association scale as functional class IV, with etiologies including ischemic heart disease, idiopathic dilated cardiomyopathy and valvular disease. They were receiving a standard triple drug therapy for immunosuppression (cyclosporine, prednisone and azathioprine). In 15 patients moderate hypertension was treated with calcium receptor antagonists. All patients were free of signs of rejection, as confirmed by endomyocardial biopsies.

Beyond the 3-week standard immediate postoperative rehabilitation, all HTRs had resumed their professional or private activities but none was enrolled in any regular physical training program at the time of the study. Twelve of them underwent a supervised 6-week endurance training program, either at the laboratory (n = 6) or at home (n = 6). The other four patients did not wish to engage in any form of physical training. Seven normal sedentary controls (5 men, 2 women), not taking any medication, were submitted to a training protocol at the same relative intensity. Patients and controls were comparable in age, height and body mass index (Table 1). All subjects gave informed consent according to a protocol approved by the local Ethics Committee.

Experimental design. Maximal exercise testing, endurance test, computed tomography (CT) of the thigh and biopsies of the vastus lateralis muscle were performed in 12 HTRs and C before and after the 6-week training period. Muscle biopsies were performed in the four nontrained HTRs in the same time interval.

Table 1. Physical Characteristics of C and HTRs

		HTR Patients		
	Normal Subjects	With Training	Without Training	p Value
n (woman/man)	7 (2/5)	12 (1/11)	4 (0/4)	
Age (years)	41 ± 13	40 ± 12	45 ± 8	0.8
Body height (cm)	173 ± 4	170 ± 8	172 ± 5	0.9
Body mass index (kg/m^2)	22.2 ± 1.7	24.9 ± 3.8	22.5 ± 2.7	0.5

Values are means ±SD. There are no significant differences among the three groups. C = age-matched control subjects; HTR = heart transplant recipients.

Maximal exercise testing. Peak oxygen uptake (peak $\dot{V}O_2$) was measured with a graded exercise test in the upright position, using an electronically braked bicycle ergometer (MEDIFIT 1000 S) and a breath-by-breath metabolic measurement chart (MEDISOFT). Cardiac frequency was measured by an electrocardiographic recorder (Schiller). The workload was increased stepwise by 20 W every 2 min from an initial workload of 20 W until volitional exhaustion. Ventilatory threshold was determinated according to Beaver et al. (14). Appropriate safety measures were taken in all tests and exercise sessions.

Training protocol. Training consisted of three sessions of 45 minutes per week for 6 weeks of modified interval training, the square wave endurance exercise proposed by Gimenez et al. (15) and adapted by Lonsdorfer et al. (16). This mode of exercise consisted of nine successive bouts of 5 min duration. During each exercise bout, a 4-min period of moderate exercise (base level) was followed by a 1-min period of heavy work (peak level). Base and peak levels were set at the individual ventilatory threshold and at 90% of maximal tolerated power, respectively. Target work rates were adjusted by maintaining the exercise heart rate during training at ~130 and 145 beats/min for base and peak levels. These rates were individually adjusted during the initial maximal exercise test. As there was no significant difference in the results of training between the group trained at home and the group trained at the laboratory, the two groups were considered as a whole.

Computed tomography (CT). CT (ELSCINT ELITE PLUS) was performed on the right thigh, exactly halfway between the major trochanter and the knee joint space. Subjects were examined in the supine position, with the thigh muscle relaxed. Radiographic factors were a cycle time of 4.2 s, 140 kV, 300 to 515 mA/s and a slice thickness of 2.5 mm. Evaluations were made at a window width of 507 and at a level between 10 and 40 to have good contrast between muscle, bone and fat. A semiautomatic electronic planimeter (MOP AM O3; Kontron, Zurich) was used to quantify the following parameters: total thigh area, thigh muscle area, quadriceps muscle area, thigh fat area, (total thigh area minus bone and muscle areas). The radiographs were analyzed blindly two times, and the estimates reported are averages of both independent measurements. The coefficient of variation for paired observations for these parameters was ~0.24% (17).

Muscle biopsy, electron microscopy and morphometry. Biopsies were taken from the same site of the vastus lateralis muscle at midthigh level under local anesthesia, using the technique of Bergström (18). The subjects were instructed not to perform any physical exercise for 24 h before the biopsy and to maintain a normal diet with regard to carbohydrates and fat. The muscle tissue samples were processed for electron microscopy by fixation in a 6.25% solution of glutaraldehyde as previously described (19). Four randomly chosen blocks from each biopsy were used for the stereological analysis. The orientation of the secretions was essentially transverse relative to the muscle fiber axis. Capillary number, fiber number and fiber cross-sectional area were estimated at a final magnification of 1,500. Five

Table 2. Benefits of Training on the Incremental Maximal Exercise Test and on the 45-Min Endurance Test

				p Values		
				Main Effects		
		Normal Subjects (n = 7)	Patients (n = 12)	Training	Group	Interaction
Peak $\dot{V}O_2$ (ml/min per kg)	Before	31.3 ± 4	22.5 ± 4.9	< 0.001	0.002	0.7
	After	33.6 ± 5.6	25.2 ± 6.2			
Ventilatory threshold $\dot{V}O_2$ (ml/min per kg)	Before	15.1 ± 0.5	13.0 ± 3.8	< 0.001	0.1	0.04
	After	20 ± 2	16.0 ± 4.3			
Total endurance work (kgJoule/kg)	Before	3.9 ± 0.9	2.2 ± 0.9	< 0.001	0.002	0.6
	After	5.1 ± 1.0	3.4 ± 0.9			

Values are means ±SD. Ventilatory threshold is expressed as $\dot{V}O_2$ normalized to body mass.

micrographs per block (20 micrographs per biopsy) were taken in consecutive frames of slotted grids (type R, 100 A, Veco Co, Amsterdam, The Netherlands) yielding >100 muscle profiles for analysis in each biopsy. A final magnification of 24,000 was used for estimation of the volumes of mitochondria and intracellular lipid droplets per unit volume of muscle fiber. Ten micrographs per block (40 micrographs per biopsy) were taken with a routine sampling procedure in consecutive frames of 200^2 mesh grids. Pictures of the 35 mm films were projected on a screen fitted with quadratic line grids. Point counting was performed with an A 100 grid (100 test points) for the lower magnification and with a B 36 grid (144 test points) for the higher magnification (19). Estimates of variables were obtained according to standard stereological procedures.

Absolute values of structural quantities were calculated as follows from the data of the CT scans and the morphometry of the biopsies: capillary length density was calculated by multiplying capillary density with a constant (1.43) characterizing the capillary tortuosity in human muscle biopsies (20). Total length of capillaries (contained in a slice of quadriceps muscle of 1 cm thickness) was obtained by multiplying the quadriceps muscle volume by the capillary length density. Total volume of mitochondria was obtained by multiplying quadriceps muscle volume by the volume density of total mitochondria.

Statistical analysis. All data are reported as mean values ±SD. Comparisons between the physical characteristics of C and HTRs were performed using a one-way analysis of variance (Systat 5.0 for Windows). Comparisons of the two trained groups (C and HTRs) before and after training were performed using two-way repeated analysis of variance (Systat 5.0 for Windows), and p values of the two main effects and of the interaction test appear in Tables 2, 3 and 4. Data from skeletal muscle biopsies before and after a 6-week period in the small group of nontrained HTR were compared using the Student *t* test for paired values.

Results

There is no significant difference for anthropometric characteristics between HTRs and C, as shown in Table 1. Body weight did not change after 6 weeks with or without training in either group, HTRs or C.

Exercise tests. Table 2 reports the changes in the group means and the significance of differences for major parameters of the exercise tests performed before and after 6 weeks of endurance training. Before training, peak $\dot{V}O_2$ of the HTRs was significantly lower (31%) than that of C. The ventilatory threshold, expressed as $\dot{V}O_2$ normalized to body mass, was not significantly different in HTRs and C. Total endurance work sustained during the 45-min training session was significantly lower by 41% in HTRs compared to C. After training, peak $\dot{V}O_2$ increased significantly in both groups, but $\dot{V}O_2$ in the HTRs remained lower than that of the control group. The ventilatory threshold also increased significantly and by the same relative amount in both groups, with a significantly lower value for the HTR group. As there is an interaction, we examined the simple effects of training within each group, which show that there is a significant increase in both groups ($p < 0.001$ for C; $p < 0.004$ for HTR). Total endurance work performed during the last training session was significantly increased by 31% for C and by 54% for HTRs. With regard to ventilatory threshold and total endurance work, HTRs after training reached values observed for untrained controls before training.

Computed tomography. Table 3 reports mean values of the CT thigh measurements before and after 6 weeks of training. No significant change was seen in the mean total cross-sectional area of the thigh nor in thigh muscle area in either group, with similar values in normal subjects and HTRs. There is a significant effect of training on the cross-sectional area of quadriceps muscle without any group effect nor interaction between the two groups. All other measured parameters did not differ in the two groups and did not change with training.

Morphometry. The following results of the biopsies of the nontrained HTR group before/after a 6-week period are not significantly modified: volume density of total mitochondria (%) 4.68 ± 1.1/3.96 ± 0.72; volume density of subsarcolemmal mitochondria (%) 0.82 ± 0.45/0.67 ± 0.24; volume density of interfibrillar mitochondria (%) 3.86 ± 0.66/3.29 ± 0.73; volume density of intracellular lipid deposits (%) 0.61 ± 0.54/0.32 ± 0.32; capillary-to-fiber ration (unitless) 1.04 ± 0.29/1.12 ± 0.40; capillary density (per mm^{-2}) 378 ± 78/384 ± 78; capillary length density (per mm/mm^3) 537 ± 104/549 ± 112; fiber cross-sectional area (μm^2) 2880 ± 1080/3000 ± 1160.

Table 3. Results of CT of the Thigh before and after 6 Weeks of Training in C and HTRs

				p Values		
				Main Effects		
		Normal Subjects (n = 7)	Patients (n = 12)	Training	Group	Interaction
Total thigh cross-sectional area (cm^2)	Before	209.8 ± 20.7	206.8 ± 46.1	0.6	0.8	0.8
	After	210.3 ± 22.9	210.1 ± 45.9			
Thigh muscle area (cm^2)	Before	138.7 ± 17.6	130.0 ± 16.8	0.5	0.2	0.9
	After	141.3 ± 17.8	133.6 ± 15.9			
M. quadriceps area (cm^2)	Before	70.7 ± 12.8	64.6 ± 7.2	0.02	0.3	0.4
	After	74.3 ± 12.6	69.8 ± 8.8			
Thigh fat area (cm^2)	Before	67.9 ± 32.5	69.1 ± 48.9	0.99	0.1	0.9
	After	67.3 ± 34.7	68.6 ± 52.6			
Fat/muscle ratio	Before	0.53 ± 0.32	0.55 ± 0.46	0.9	0.2	0.9
	After	0.52 ± 0.33	0.54 ± 0.50			

Values are means ±SD. Abbreviations as in Table 1.

Table 4 presents mean values of the structural variables estimated with the morphometric techniques in the two trained groups. Initial values of mitochondria are not different among the two groups. After the 6-week training period, the volume density of total mitochondria and its subdivisions, central and subsarcolemmal volume densities, are significantly increased in the two trained groups, with no significant difference between controls and trained HTRs. The volume density of lipid droplets is similar in the two groups and is not significantly modified by training. Capillary-to-fiber ratio is not different between the two groups before training; it increases significantly after training as a main effect. Because the test of interaction is significant, we examined the simple effects of training within each group, which show a significant increase in C ($p = 0.008$) and not in HTRs ($p = 0.1$). Capillary density and capillary length density are significantly reduced in the HTR group before training as compared to C. Both of these indexes are unmodified by training in either group, though they are significantly higher in the normal subjects. The fiber cross-sectional area is similar in the two groups and is not modified by training.

To get an estimation of the total quadriceps muscle content of structural components, volume density of total mitochondria and capillary length density were multiplied by the quadriceps muscle area assessed by CT in the two trained groups. The values appear in Figure 1. Total volume of mitochondria, similar in C and HTRs before training, increases significantly in both groups, by 38% for HTRs and 34% for C. Total capillary length is significantly decreased in HTRs before training and is not significantly modified by training in either group.

Table 4. Morphometry of Muscle Ultrastructure

				p Values		
				Main Effects		
		Normal Subjects (n = 7)	Patients (n = 12)	Training	Group	Interaction
Volume density of total mitochondria (%)	Before	4.58 ± 0.77	4.33 ± 1.22	< 0.001	0.3	0.4
	After	6.09 ± 0.78	5.47 ± 1.13			
Volume density of subsarcolemmal mitochondria (%)	Before	0.88 ± 0.37	0.72 ± 0.44	0.004	0.2	0.8
	After	1.5 ± 0.5	1.25 ± 0.63			
Volume density of interfibrillar mitochondria (%)	Before	3.71 ± 0.50	3.61 ± 0.84	< 0.001	0.4	0.3
	After	4.60 ± 0.51	4.21 ± 0.81			
Volume density of intracellular lipid deposits (%)	Before	0.63 ± 0.45	0.51 ± 0.20	0.1	0.7	0.5
	After	0.74 ± 0.52	0.76 ± 0.51			
Capillary-to-fiber ratio (unitless)	Before	1.38 ± 0.34	1.14 ± 0.32	0.006	0.07	0.04
	After	1.64 ± 0.45	1.25 ± 0.38			
Capillary density (mm^2)	Before	437 ± 115	352 ± 77	0.3	0.02	0.8
	After	460 ± 92	369 ± 60			
Capillary length density (mm/mm^3)	Before	625 ± 164	503 ± 110	0.3	0.02	0.8
	After	658 ± 132	528 ± 86			
Fiber cross-sectional area (μm^2)	Before	3240 ± 630	3240 ± 670	0.1	0.7	0.5
	After	3570 ± 630	3380 ± 830			

Values are means ± SD.

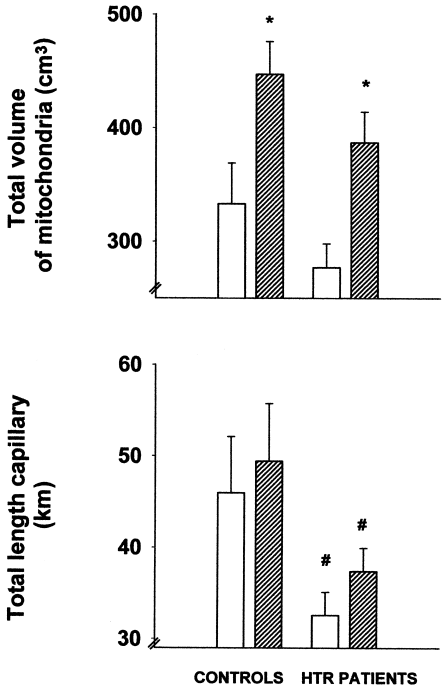

Figure 1. Total volume of mitochondria and total capillary length in the quadriceps muscle of C and HTR patients before and after a 6-week training program. **Open bars** = pretraining; **hatched bars** = post-training. *$p < 0.05$ for comparisons before and after training within each group. #$p < 0.05$ for comparisons between C and HTR patients.

Discussion

Several studies have demonstrated the beneficial effects of a training program on cardiovascular, respiratory and metabolic parameters in HTRs (1,2,3,9,10,21). In a previous study (11), we reported the unexpected result that 1 year after grafting, there was no qualitative or quantitative difference between the volume density of mitochondria in the vastus lateralis muscle of nontrained HTRs compared to normal sedentary controls. The major structural difference between the two groups concerned the capillary density and the capillary-to-fiber ratio, which were both significantly decreased in HTRs. Because deconditioning was one of the mechanisms potentially responsible for this observation, it seemed important to appraise the changes induced by endurance training on the skeletal muscle structure of these patients. To test the efficiency of the training program, we included a group of normal sedentary comparable volunteers (C) training at the same relative intensities. To ascertain that the observed changes were not due to spontaneous recovery, we included biopsies taken at 6-week intervals from a group of four inactive HTRs.

Changes in general aerobic performance. A total of only 14 h of exercise during 6 weeks resulted in a mean increase of 11% in peak $\dot{V}O_2$. This is somewhat less than the 18% increase obtained by HTR patients after a 10-week program (3). The difference can be attributed to the fact that the patients in the study of Keteyian et al. (3) were initially less fit than our patients (initial peak $\dot{V}O_2$: 16 ml/min per kg), and the exercise training program of the former group was more intense, that is, more muscle mass was mobilized by several types of exercise like rowing, treadmill and arm and leg exercises. For the C, our results do not differ from the mean increase of 14% of peak $\dot{V}O_2$ obtained after 6 weeks (15 h) of strenuous cycle training in normal sedentary subjects by Hoppeler et al. (22).

We believe that this training program corresponds to a reasonable rehabilitation practice routinely feasible either in institution or at home. Indeed, we expected the benefits of this program to be lower than in that of Kavanagh et al. (1), because the 18 months of intense training in that program does not correspond to what can be routinely obtained from the average HTRs.

Significantly more relevant than the peak $\dot{V}O_2$ is the shift of the ventilatory threshold observed in both groups, which is believed to reflect an increase in tissue oxidative capacity. Of note also is the important increase of 31% (C) and 54% (HTRs) of the total endurance work performed in one session, when comparing the first to the last training session.

Structural changes. None of the structural markers analyzed was spontaneously modified after 6 weeks in the nontraining group. Thus the changes observed in controls and in HTRs can be attributed to the short endurance training program we implemented.

Muscle volume. We did not observe any significant change in thigh muscle cross-sectional area after training in either group, nor differences between C and HTRs. This observation contrasts with the findings by Horber et al. (17), who described a significant increase of the midthigh muscle area after 7 weeks of isokinetic training in nine renal transplant patients receiving similar doses of glucocorticoids and azathioprine. This difference must likely be attributed to the more strength-oriented isokinetic training protocol. It also contrasts with the increase of lean tissue mass described by Kavanagh et al. (1) among 36 HTRs subjected to a long-lasting endurance training protocol involving a larger number of muscles.

Mitochondria. The predominant effect of our endurance training on skeletal muscle structure is the marked increase in volume density of total mitochondria (33% for C, 26% for HTRs; $p < 0.001$). This result is comparable with that of a previous study on normal sedentary subjects, who showed a 40% increase in total mitochondria after 15 h of cycling at 80% of the peak $\dot{V}O_2$ (22). The initial mitochondrial volume densities were not lower in HTRs than in C as might have been expected from a previous study on patients with heart failure (23) but confirms our previous findings (11). The increase in total volume of mitochondria is brought about by both an increase in volume density of mitochondria as well as an increase in muscle volume.

Our results show a predominant increase of subsarcolemmal mitochondrial volume density (74% in HTRs, 70% in C) and a smaller increase of interfibrillar mitochondria (16% in HTRs, 24% in C). These results are in accordance with previous findings in normal subjects (24) and in patients with

renal transplants (17) and confirm previous data suggesting that preferential proliferation of subsarcolemmal mitochondria can be related to a better use of blood-borne substrate following endurance exercise (25). The increase of the lipid droplet volume density did not reach the limit of significance in any of the trained groups. This is usually seen in endurance training protocols of similar duration in normal subjects (22).

It is noteworthy that there is a significant relationship between the change in volume density of total mitochondria of HTRs and both the change in their peak $\dot{V}O_2$ ($r = 0.63$, $p = 0.03$) and the change in their $\dot{V}O_2$ at ventilatory threshold ($r = 0.60$, $p = 0.03$). These results are in accordance with those shown by Hambrecht et al. (26) on muscle biopsies in patients with heart failure subjected to a 6-month training program. Nevertheless, the increase in mitochondrial content did not normalize the $\dot{V}O_2$ of the HTRs. As already noted (11), mitochondrial function might be impaired by cyclosporine treatment (12,13).

Capillary supply. Generally, skeletal muscles show adaptation to aerobic training by an increase in the number of their capillaries, which leads to a reduction of the diffusion distance for gases and improves conditions for substrate transfer (27). This is the case for C but not for HTRs. The capillary-to-fiber ratio increases significantly by 19% in C. This is somewhat less than the 28% increase in the study of Ingjer (28) and the 26% increase in the study of Hoppeler et al. (22), both measured in normal subjects. Because the fiber cross-sectional area is also increased in C, though not significantly, capillary density and hence capillary length density remain constant. For this last parameter our results are in close agreement with those of Drexler et al. (23) in normal subjects (654 ± 141 in Drexler's study versus 625 ± 164 mm/mm^3 C in this study).

Compared to previous studies, the relatively small increase observed in C can be linked to the lower work load of our endurance training protocol (~50% to 60% of $\dot{V}O_2$ versus 80% of $\dot{V}O_2$ for the subjects of Hoppeler et al. (22).

The most striking findings of our study are the observation of the significantly reduced number of capillaries of these patients in the pre- and post-training state compared to C and the lack of a significant training-induced increase of muscular capillaries in HTRs. This suggests that a lack of O_2 supply could limit the exercise capacity of HTRs and, further, that the lack of angiogenesis could potentially limit the effectiveness of exercise training. The smaller extent of the capillary network could be due to several factors. In patients with heart failure, an impaired vasodilatory response to exercise is well-documented. An increase of neurohumoral vasoconstrictive forces (29) and abnormalities of flow-dependent endothelium-mediated vasodilatation have been involved (30). These abnormalities might have persisted after transplantation, though a recent study (31) suggests that endothelium-dependent vasodilatation is at least partially restored after grafting, as is maximal vasodilator response (32). Moreover, cyclosporine has been shown to produce vasoconstriction in renal circulation (33) with significant reduction of glomerular capillaries in renal transplant biopsies (34). Other mechanisms explaining this lack of muscular capillaries and exercise-induced angiogenesis in HTRs must be evoked. Vascular endothelial growth factor (VEGF) has been recently described as a well-characterized direct-acting angiogenic factor (35). Using an in vitro model, Pepper et al. (36) have shown that the cytokine transforming growth factor-beta (TGF-β) exerts a biphasic effect on VEGF, potentiating effect at low concentration and inhibiting effect at relatively high concentration. As cyclosporine is known to stimulate TGF-β (37), it can be hypothesized that high doses of TGF-β would decrease VEGF and thus reduce the muscular capillary density and block the training-induced formation of muscular capillaries in HTRs. This hypothesis could be tested in a prospective study.

Conclusions

1. Exercise training in HTRs and C leads to similar improvements of central and peripheral factors of aerobic work capacity.
2. Mitochondrial volume density is normal in HTRs with a reduced exercise tolerance and increases with exercise training.
3. A training response of capillaries could be observed only in C and not in HTRs. The training-induced changes were relatively small, probably due to the low intensity of training as compared with that in other studies.
4. The discrepancy between a decreased capillary supply and a normal mitochondrial complement previously described in the HTRs was accentuated after training. Capillary deficiency is likely not caused by the lack of exercise but may be due to the toxicity of immunosuppressive drugs, especially cyclosporine.

We express sincere gratitude to Helgard Claassen, Eric Epailly, Jean Durckel, Sylvie Beyhurst, Mireille Trautmann, Françoi Piquard and Arthur Pape for excellent and skillfull assistance.

References

1. Kavanagh T, Yacoub MH, Mertens DJ, Kennedy J, Campbell RB, Sawyer P. Cardiorespiratory response to exercise training after orthotopic cardiac transplantation. Circulation 1988;77:162–71.
2. Squires RW. Exercise training after transplantation. Med Sci Sports Exerc 1991;23:686–94.
3. Keteyian S, Shepard R, Ehrman J, et al. Cardiovascular responses of heart transplant patients to exercise training. J Appl Physiol 1991;70:2627–31.
4. Savin WM, Haskell WL, Schroeder JS, Stinson EB. Cardiorespiratory responses of cardiac transplant patients to graded, symptom-limited exercise. Circulation 1980;62:55–60.
5. Kao AC, Van Trigt III P, Shaeffer-McCall GS, et al. Central and peripheral limitations to upright exercise in untrained cardiac transplant recipients. Circulation 1994;89:2605–15.
6. Meyer M, Cerretelli P, Marconi C, Rieu M, Cabrol C. Cardiorespiratory adjustment to exercise after cardiac transplantation. In: Reinhart KR, Eyrich K, eds. Clinical Aspects of O_2 Transport and Tissue Oxygenation. Berlin: Springer-Verlag, 1990;477–97.
7. Stratton JR, Kemp GJ, Daly RC, Yacoub M, Rajagopalan B. Effects of cardiac transplantation on bioenergetic abnormalities of skeletal muscle in congestive heart failure. Circulation 1994;89:1624–31.

8. Mettauer B, Lampert E, Petitjean P, et al. Persistent exercise intolerance following cardiac transplantation despite normal oxygen transport. Int J Sports Med 1996;17:277–86.
9. Badenhop DT. The therapeutic role of exercise in patients with orthotopic heart transplants. Med Sci Sports Exerc 1995;27:975–85.
10. Lampert E, Oyono-Enguelle S, Mettauer B, Freund H, Lonsdorfer J. Short endurance training improves lactate removal ability in patients with heart transplants. Med Sci Sports Exerc 1996;28:801–7.
11. Lampert E, Mettauer B, Hoppeler H, Charloux A, Charpentier A, Lonsdorfer J. Structure of skeletal muscle in heart transplant recipients. J Am Coll Cardiol 1996;28:980–4.
12. Hokanson JF, Mercier JG, Brooks GA. Cyclosporine A decreases rat skeletal muscle mitochondrial respiration in vitro. Am J Respir Crit Care Med 1995;151:1848–51.
13. Mercier JG, Hokanson JF, Brooks GA. Effects of cyclosporine A on skeletal muscle mitochondrial respiration and endurance time in rats. Am J Respir Crit Care Med 1995;151:1532–6.
14. Beaver WL, Wassermann K, Whipp BJ. A new method for detecting anaerobic threshold by gas exchange. J Appl Physiol 1986;60:2020–7.
15. Gimenez M, Servera E, Salinas W. Square wave endurance exercise test for training and assessment in trained and untrained subjects. Eur J Appl Physiol Occup Physiol 1982;49:359–87.
16. Lonsdorfer J, Lampert E, Mettauer B, et al. Physical fitness after cardiac transplantation: a proposal for an endurance training program and its assessment. Sci Sports 1992;7:39–44.
17. Horber FF, Hoppeler H, Scheidegger JR, Grunig BE, Howald H. Impact of physical training on midthigh muscle in normal subjects and in patients treated with glucocorticoids. J Clin Invest 1987;79:1181–90.
18. Bergström J. Muscle electrolytes in man. Scand J Clin Lab Invest 1962;14 (suppl 68):11–2.
19. Hoppeler H, Lüthi P, Claassen H, Weibel ER, Howald H. The ultrastructure of the normal human skeletal muscle. Pfluegers Arch 1973;344:217–32.
20. Hoppeler H, Kayar SR. Capillarity and oxidative capacity of muscles. News Physiol Sci NIPS 1988;3:113–6.
21. Niset G, Hermans L, Depelchin P. Exercise and heart transplantation: a review. Sports Med 1991;12:359–79.
22. Hoppeler H, Howald H, Conley K, et al. Endurance training in humans: aerobic capacity and structure of skeletal muscle. J Appl Physiol 1985;59: 320–7.
23. Drexler H, Riede U, Münzel T, König H, Funke E, Just H. Alterations of skeletal muscle in chronic heart failure. Circulation 1992;85:1751–9.
24. Howald H, Hoppeler H, Claassen H, Mathieu O, Straub R. Influences of endurance training on the ultrastructural composition of the different muscle fibre types in humans. Pfluegers Arch 1985;403:369–76.
25. Kiessling KH, Pilström L, Bylund AC, Saltin B, Piehl K. Enzyme activities and morphometry in skeletal muscle of middle-aged men after training. Scand J Clin Lab Invest 1974;33:63–9.
26. Hambrecht R, Niebauer J, Fiehn E, et al. Physical training in patients with stable chronic heart failure: effects on cardiorespiratory fitness and ultrastructural abnormalities of leg muscles. J Am Coll Cardiol 1995;25:1239–49.
27. Saltin B, Gollnick PD. Skeletal muscle adaptability: significance for metabolism and performance. In Handbook of Physiology. Skeletal Muscle. Bethesda: Am Physiol Soc 1993;10:555–631.
28. Ingjer F. Effects of endurance training on muscle fibre ATP-ase activity, capillary supply and mitochondrial content in man. J Physiol Lond 1979;294: 419–32.
29. Cavero PG, Sudhir K, Galli FC, De Marco T, Chatterjee K. Stress-induced changes in forearm vascular dynamics in heart failure: distinction between ischemic and dilated cardiomyopathy. (abstr.) J Am Coll Cardiol 1992;3: 316A.
30. Kubo SH, Rector TS, Bank AJ, Williams RE, Heifetz SM. Endothelium dependent vasodilatation is attenuated in patients with heart failure. Circulation 1991;84:1589–96.
31. Kubo SH, Rector TS, Banks AJ, et al. Effects of cardiac transplantation on endothelium dependent dilatation of peripheral vasculature in patients with congestive heart failure. Am J Cardiol 1993;71:88–93.
32. Sinoway LI, Minotti JR, Davis D, et al. Delayed reversal of impaired vasodilatation in congestive heart failure after cardiac transplantation. Am J Cardiol 1988;61:1076–9.
33. Kaye D, Thompson J, Jennings G, Esler M. Cyclosporine therapy after cardiac transplantation causes hypertension and renal vasoconstriction without sympathetic activation. Circulation 1993;88:1101–9.
34. Furness PN, Morgan J, Horsburgh T. Glomerular perfusion in renal allograft biopsies. Histopathology 1993;22:65–8.
35. Klagbsbrun M, D'Amore PA. Regulators of angiogenesis. Ann Rev Physiol 1991;53:217–39.
36. Pepper MS, Vassali JD, Orci L, Montesano R. Biphasic effect of transforming growth factor-1 on in vitro angiogenesis. Exp Cell Res 1993;204:356–63.
37. Shin GT, Khanna A, Sharma VK, Ding R, Azizlerli S, Suthanthiran M. In vivo hyperexpression of transforming growth factor beta 1 in humans: stimulation by cyclosporine. Transpl Proc 1997;29:284.